AFFIDAVIT OF RANDOLPH J. NOELLE Ph.D.

I, Randolph J. Noelle, declare and state as follows:

(1) I am an inventor of the above-identified application.

(2) I am a Professor of Immunology at Dartmouth College.

(3) I have substantial expertise in the area of immunological/therapeutic research, and specifically in the area of gp39 (CD154). My status as an expert is substantiated by the attached curriculum vitae.

(4) I have reviewed the most recent Office Action issued by the European Patent Office on February 13, 1998. Based thereon, it is my understanding that the Examiner has concluded that the antibody which is the subject of the claims of this application, namely the 24-31 antibody and antibodies having the same epitopic binding specificity, is not inventive over the 5c8 antibody of Lederman et al. This antibody is disclosed in the following references which are cited in the Office Action: Lederman et al, *J. Exp. Med.*, Vol. 175:1071-1101 (April 1992) (D1); Lederman et al, Vol. 149:3817-3826 (Dec. 1992) (D2); and Marshall et al, *J. Clin. Immunology*, Vol. 13(3):165-174 (1993) (D3).

(5) I note that the Examiner asserts in the Office Action that "the mab 5c8 (subtype IgG2a) as disclosed in e.g., D1, D3 and D3 is also an anti-human gp39 monoclonal antibody

being merely produced by another hybridoma cell line." Moreover, it is my further understanding that the Examiner has suggested that the rejection can be obviated upon a demonstration that the 24-31 exhibits "advantageous and unexpected results" in relation to the 5c8 antibody.

(7) That I have conducted the following comparisons which I believe demonstrate that the 5c8 and 24-31 antibodies, while both being murine anti-human gp39 (CD154) antibodies, do not function equivalently. To the contrary, 24-31 exhibits greater capacity to block CD40-gp39 binding, and enhanced ability to neutralize the biological activity of gp39 (CD154). Accordingly, this antibody possesses advantages and unexpected results in relation to 5c8. These experiments are discussed in detail below.

(8) A first experiment was conducted wherein I compared the binding of 24-31 and 5c8 to immobilized soluble CD154 (sCD154). In this experiment ELISA plates were coated with sCD154 at a concentration of 5 μg/ml. The sCD154 comprised a recombinant, soluble form of CD154 fused to CD8 and the extracellular domain of CD154. The binding of biotinylated (bio) 5c8, bio-24-31 and bio-CD40-Ig (the receptor for CD154) to said sCD154 were then compared. The results of this experiment are contained in Figure 1 (attached hereto). It can be seen therefrom that 5c8 and 24-31 bound with similar affinity to sCD154,

with 24-31 having a slightly lower binding affinity. By contrast, bio-CD40 Ig bind to sCD154 at a much lower affinity.

(9) A second experiment was conducted wherein I compared the capability of 5c8 and 24-31 to compete with CD40 for binding soluble sCD154. ELISA plates were again coated with sCD154 at a concentration of 5 μg/ml. CD40Ig-Biotin was then introduced at a concentration of 10 μg/ml and allowed to compete for binding to sCD154 with non-conjugated 5c8, 24-31, CD40Ig and a control antibody (CmIgG1) added at concentrations ranging from 0 to 10 μg/ml. These results are contained in Figure 2 (also attached). Based thereon, it can be seen that 24-31 and 5c8 behave similarly in their ability to compete with CD40 in binding sCD154.

(10) A third experiment was conducted to determine whether that the binding sites (epitopes) to which 24-31 and 5c8 bind on the CD154 molecule are distinct. This experiment actively involved two cross-competition experiments. CD8-hCD154 was again used to coat microtiter wells at a concentration of 5 μg/ml. In the first part of this experiment, the ability of non-conjugated 5c8, 24-31 or control CmIgG2A to compete with 5c8 for binding to CD154 was compared. These results are contained in Figure 3 (attached). In the second part of this experiment, the binding of biotinylated 24-31 at a concentration of 10 μg/ml to a similarly coated CD8-hCD154 microtiter well, was competed with non-conjugated 5c8, 24-31, or

control CmIgG1. These results are contained in Figure 4 (attached). In comparing the results of this experiment, which use sCD154 as a target, it can be seen that 24-31 was more effective at inhibiting the binding of bio-5c8 to sCD154, then 5c8 was at inhibiting the binding of bio-5c8 to sCD154 (Figure 3). By contrast, the reverse competition experiment indicated that 5c8 was far less effective at inhibiting the binding of bio-24-31 to sCD154 than 24-31 was at inhibiting such binding. (Figure 4). Moreover, it can further be seen that at high 24-31 concentrations, the binding of 5c8 and 24-31 are completely inhibited. By contrast, at high 5c8 concentrations, epitopes for 24-31 are still available for binding. These results support a conclusion that 5c8 and 24-31 do not bind the same epitope on CD154. However, the fact that these antibodies do compete with one another for binding to sCD154 would suggest that these antibodies may bind to epitopes on sCD154 that are proximate to each other.

(11) Another experiment was conducted wherein the effects of 5c8 and 24-31 on a cell line expressing hCD154 were compared. In this experiment CHO cells which expressed CD154 on their surface were incubated with 5c8, 24-31 and CmIgG1 and the binding of biotinylated CD40-Ig assessed by flow cytometry. The results were quantified based on the mean fluorescence intensity (average binding) of the biotinylated -CD40Ig to these cells. This experiment was conducted because it is uncertain whether the structure and serological reactivity of sCD154 is identical to the native, integral membrane form of CD154. This is

important as the *in vivo* efficacy of a blocking antibody will depend upon its capacity to bind native CD154 and block receptor binding. Therefore, to determine whether 24-31 has an increased capacity to block CD40 binding, the blocking of CD40 binding by 24-31 and 5c8 was assessed using the native, integral membrane form of CD154 (using full length CD154 expressed in Chinese Hamster Ovary cells). The results of this experiment are contained in Figure 5. It can be seen therefrom that 24-31 blocked the binding of CD40-Ig to native CD154 much more efficiently than did 5c8 (half-maximal blocking respectively of 1.5 μg/ml and 2.5 μg/ml).

(12) I also conducted another experiment wherein the capacity of 24-31 and 5c8 to inhibit the biological function of CD154 were compared. In this experiment, human peripheral blood B cells were cultured with sCD154 and IL4 for three days to induce proliferation. Titered quantities of purified 5c8 and 24-31 were then added to the culture to inhibit proliferation. Proliferation was assessed by measuring the incorporation of ^{3}H-thymidine. More specifically, to assess the capacity of 24-31 and 5c8 to block CD154 biological activity, titered quantities of 24-31 and 5c8 were introduced in culture with human B cells, sCD154 and IL4 because human B cells proliferate in response to sCD154 and IL4. Therefore, the inhibition of B-cell proliferation is a stringent functional test for evaluating the neutralizing activity of the subject anti-gp39 antibodies. It can be seen from Figure 6 that 24-31 inhibited

half-maximal B cell proliferation at approximately 0.16 μg/ml. By contrast, substantially higher ($\approx$ 3-fold) concentrations of 5c8 (0.45 μg/ml) were required to achieve similar levels of inhibition. I believe that these results provide further evidence as to the enhanced capacity of 24-31 (relative to 5c8) to inhibit soluble receptor binding.

(13) In summary, in my opinion, the results of the above experiments support the following conclusions:

(i) that 5c8 and 24-31 bind different epitopes on human CD154 (gp39);

(ii) that 5c8 and 24-31 bind to sCD154 with similar binding affinity, with 5c8 having a slightly better binding affinity;

(iii) that 24-31 inhibits the binding of gp39 (CD154)/CD40 binding more effectively than 5c8; and

(iv) that 24-31 inhibits (neutralizes) the biological function of CD154 (gp39) substantially better (about 3-fold) than 5c8.

Based thereon, it is my opinion that an antibody having the same epitopic binding specificity as 24-31, and possessing substantially the same binding affinity, does not function equivalently to the 5c8 antibody disclosed in the cited references. To the contrary, the above results indicate that the subject antibody should more effectively inhibit the binding of CD154 (gp39) to CD40 *in vivo*. Also, such antibody should better neutralize the biological function

of CD154 *in vivo*. Accordingly, I am of the opinion that the subject 24-31 antibody and antibodies having the same binding properties, should provide a more effective therapeutic agent than 5c8. This is believed to be "an advantageous and unexpected result" not suggested by the cited references (D1-D3).

(14) I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

7/26/98
Date

Randolph J. Noelle, Ph.D.

Figure #1

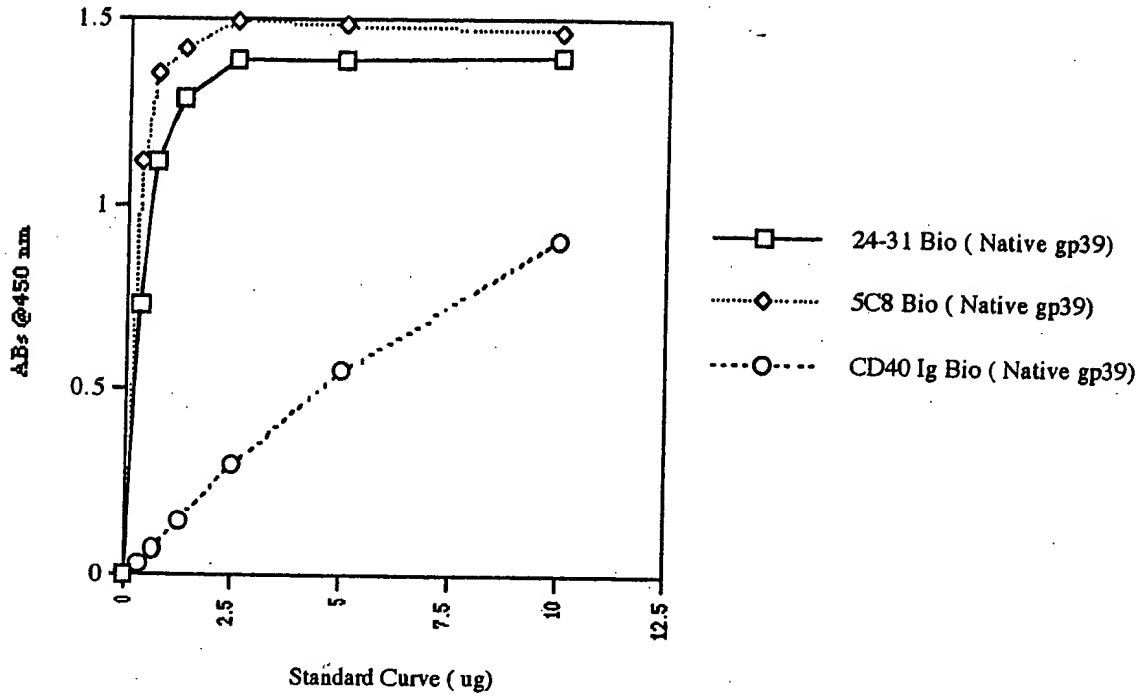

Figure 1. Comparisons of 5c8, 24-31 and CD40Ig binding to sCD154. ELISA plates were coated with sCD154 (5ug/ml) and concentrations of bio-5c8, 24-31 or CD40Ig were incubated. Detection of the bound bio-mab was detected with avidin-peroxidase.

Figure #2

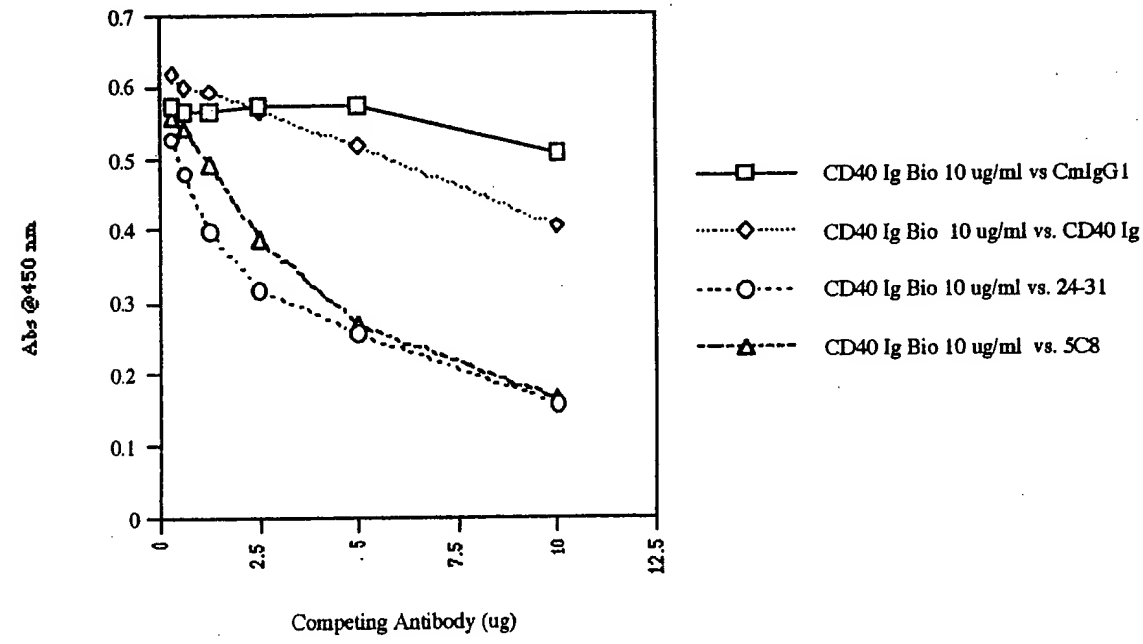

Figure 2. Comparative capacity of 5c8 and 24-31 to block CD40 binding to sCD154. ELISA plates were coated with sCD154. CD40Ig -Biotin was used at a concentration of 10 ug/ml and competed with non conjugated 5C8, 24-31, CD40 Ig, or control CmIgG1 at concentrations from 0-10 ug/ml.

Figure #3

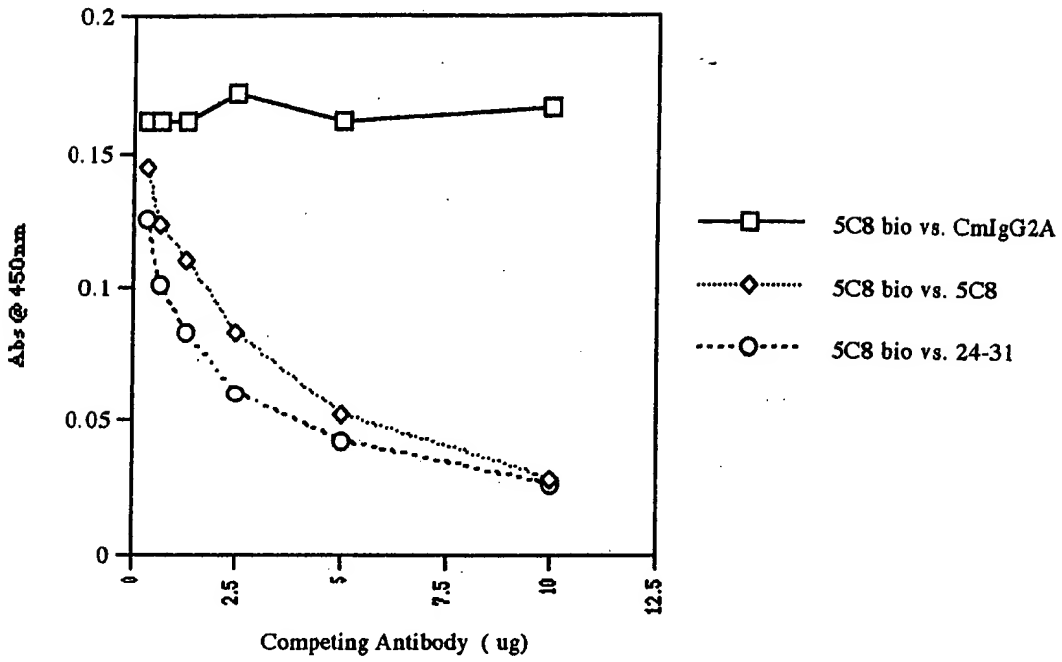

Figure 3. Blocking the binding of 5c8 by 5c8 or 24-31. CD8-hCD154 was coated at 5ug/ml to microtiter wells. The binding of 5C8 Ig Biotin at 10 ug/ml was competing with non conjugate 5C8, 24-31, or control cMIgG2A.

Figure #4

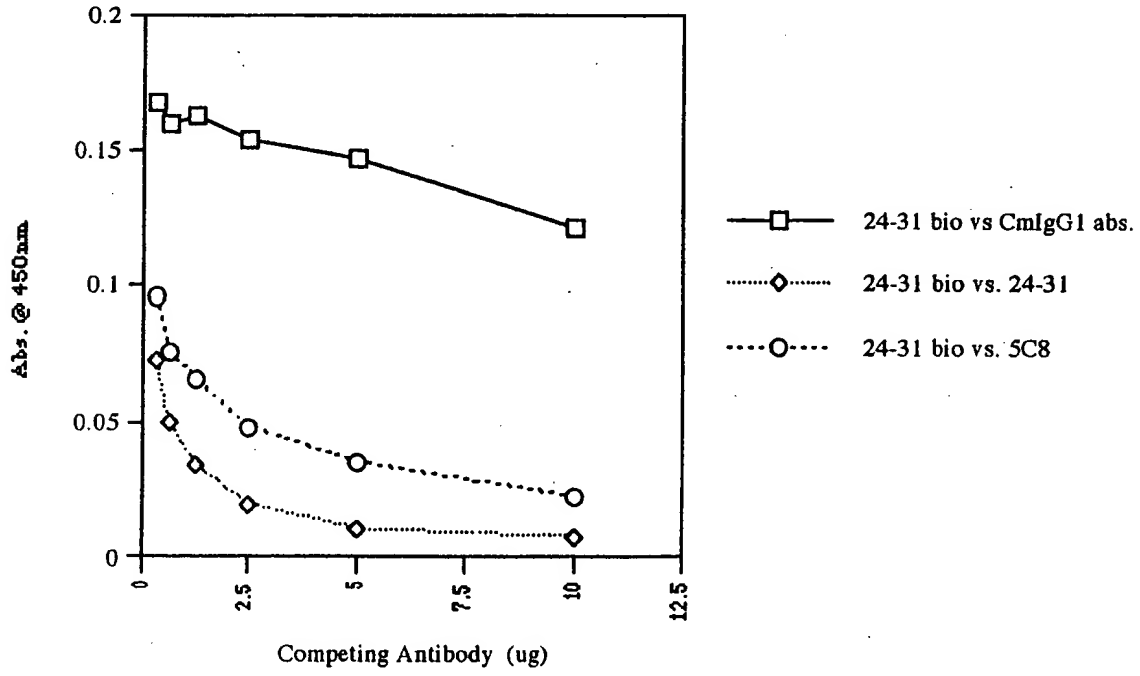

Figure 4. Blocking the binding of 24-31 by 5c8 or 24-31. CD8-hCD154 was coated at 5ug/ml to microtiter wells. The binding of 24-31 Ig Biotin at 10 ug/ml was competing with non conjugate 5C8, 24-31, or control cMIgG1

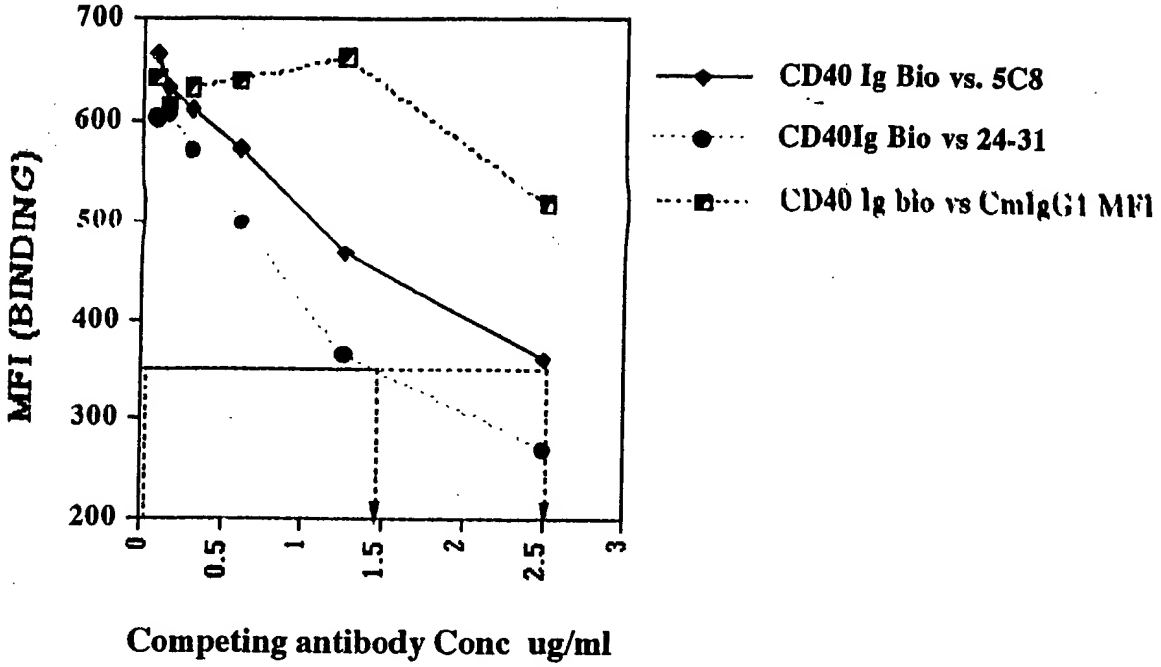

Figure 5. Blocking of CD40-Ig binding to CD154-expressing CHO cells. CD154-expressing CHO cells were incubated with the antibodies noted and then the binding of CD40-Ig-bio was assessed by flow cytometry. Results show the mean fluorescence intensity (average binding) of the bio-CD40-Ig to cells.

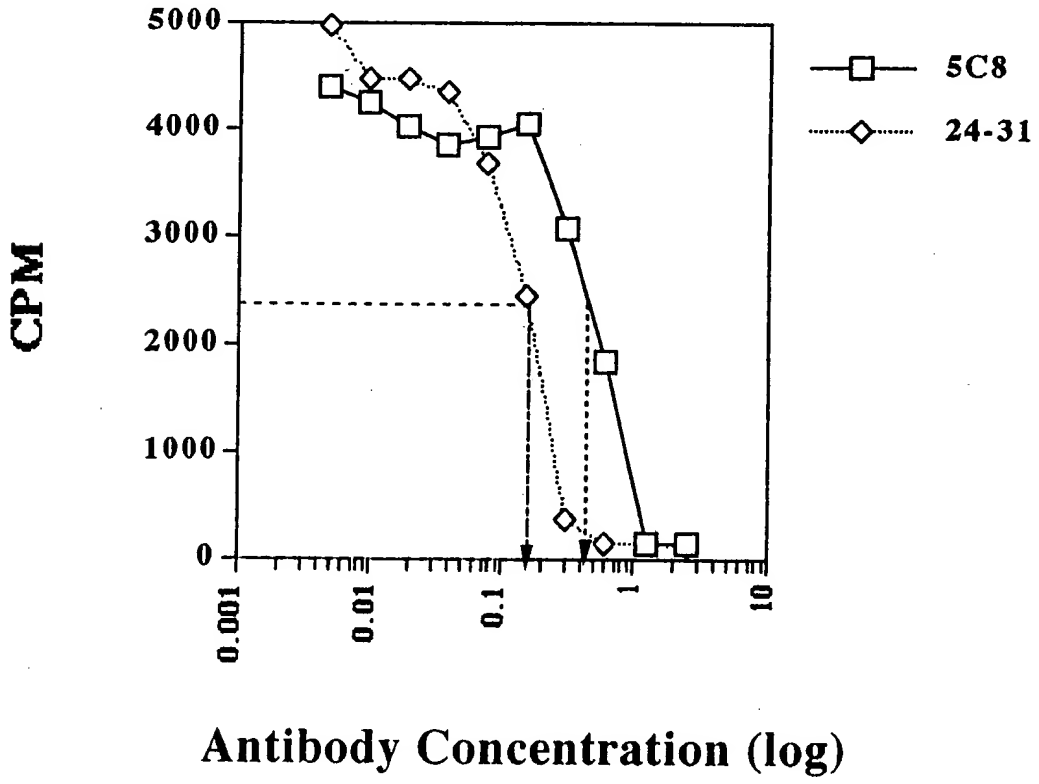

Figure 6. 24-31 half-maximally inhibits CD154-induced B cell proliferation at lower concentrations than 5c8. Human peripheral blood B cells were cultured with sCD154 and IL4 for three days to induce proliferation. Titered quantities of purified 5c8 and 24-31 were added to culture to inhibit proliferation. Proliferation was assessed by the incorporation of ^{3}H-thymidine.